Phyllosphere and Rhizosphere studies using the ALS-IR Beamline 1.4.3

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Scientific goals of Program -- To develop technology for the non-destructive identification, and high-resolution spatial mapping of rhizosphere exudates from intact plant surfaces in response to abiotic stresses, or evolved for microbial recruitment.

Impact of Results -- Considerable land area in the developing world suffers from severe soil acidity (Rao et al. 1993), nutrient deficiencies, while in industrialized countries, metal toxicities are common in soils near former manufacturing sites. IR spectromicroscopy offers an unprecedented glimpse at a significant "sink" for carbon within plants, as well as offering a new screening "tool" with which to evaluate existing germ plasm, or transgenically altered plants. The global importance of root exudates can be appreciated from the following brief examples:

(1) Acid rain, 'Waldsteben' and metal toxicity -- in both the NE United States and Eastern Europe, significant acreage of forests have been weakened or destroyed by acidification of the soil arising from elevated wet/dry deposition of sulfur dioxide and nitric oxides in industrialized areas. At normal soil pH's, aluminum is a very common but insoluble metal in most soils. When the soil pH drops <4 however, its solubility (and hence availability to plants) increases dramatically. One of the principal components of natural waters that limits the bioavailability of Al^{3+} is dissolved organic carbon (Sposito 1989), some of which is derived from root exudation. One key exudate strategy used by Al^{3+}-tolerant plants is malic acid secretion. Using the ALS, we have proposed to set-up a screening program to search for patterns in the distribution of this root-exudation strategy among plant species for use in tropical grassland restoration.

In response to industrial pollution from the transition metal Nickel, researchers have demonstrated that some plants are capable of "detoxifying" high soil burdens by the over-production of histidine-derived non-protein amino acids. In conjunction with the "rhizobox", the IR microscope has the potential has a rapid screening tool for comparison within a phylogenetic group. For plant ecologists, we then have a quantitative tool with which to address evolutionary questions: " How widespread is this mechanism within a plant family? ", or " How many times did this trait arise?" (Monson 1995; Raab et al. 1999).

(2) Nitrogen fixation and nodulation -- Nitrogen is an essential component of healthy soils and plants, and one of the key routes by which nitrogen is introduced into natural ecosystems is through the process of N_2 fixation. In a well-characterized symbiosis between legumes (members of the bean family) and soil bacteria of the genus Rhizobium, plants are able to utilize atmospheric N_2 directly for production of amino acids and proteins by forming bacterial "nodules" on the roots. In marginal wet and dry tropical locations, beans are the principal source of protein in human diets (Rao et al. 1993). In the early stages of the nodulation process, unusual flavonoid compounds are excreted from the root zones of legumes (Peters and Long 1988). These "signal" molecules serve as chemo-attractants for soil bacteria participating in the symbiosis. Only a few of the actual signal molecules have been identified, and it is of considerable interest to modern agriculture to identify these "semio-chemicals" in order to "tune" the infecting rhizobial bacteria.

(3) Soil-nutrient mobilization -- A variety of plant species have been demonstrated to excrete significant quantities of low molecular weight organic acids in response to nutrient deficiencies (Lipton et al. 1987; Dinkelaker et al. 1989; Ae et al. 1990). Second only to nitrogen (see above) in its importance to plant and human health, phosphorus is one of the most difficult nutrients for plants to acquire. Taking an evolutionary perspective, botanists now recognize this as a driving force in the creation of plant-fungal symbioses (i.e. mycorrhizae) on several occasions in the evolution of higher plants on Earth. While these fungal symbioses have significantly extended the ecological "latitudes" of higher plants, not all plant families are competent to initiate such symbioses. These plants must devise other methods to (1) increase the specific surface are of their root systems (Schmidt and Stewart 1997), (2) solubilize nutrients within their rhizospheres (Lipton et al. 1987; Dinkelaker et al. 1989; Jones et al. 1994) and (3) change the pH within the root zone (Blanchar and Lipton 1986).

Rationale for using the Synchrotron -- The 10-micron spot-size of the IR microscope at Beamline 1.4.3 allows an order-of-magnitude improvement in the fine-scale mapping of exudate gradients around plant roots. The Nicolet software that governs the collection of IR spectra through the microscope has two modes: i.) a line scan, and a ii.) 2-D array map. It might be appreciated that a linear scan oriented normal to the root surface potentially allows the mapping of gradients of root exudates produced in response to one (or more) of the above abiotic stressors. We have in fact begun collecting such spectral scans from intact roots of the legume Lens culinaris L. (mung beans) grown under phosphorus deprivation (Figure 1). The rhizosphere is the area (< 2 mm separation) surrounding the roots where microbes and plant together greatly influence the soil solution chemistry compared to the bulk soil. The fine spatial resolution of the ALS-FTIR allows us to divide this region of influence into smaller parts, and increases the accuracy with which both carbon-sources and -gradients may be delineated.

With line scans oriented perpendicularly to the leaf vasculature, the IR spectra demonstrate strong compositional gradients for leaf protein (Figure 2), based on the intense amide absorptions from 1700 - 1200 cm-1. Depending on the apparent "skin depth" of the IR radiation, we may be sampling to a depth of 10-20 microns into the photosynthetic apparatus. Significant stores of phosphoanhydride absorptions at 2200 cm-1 are also seen on the abaxial side of the leaf, whereas, on the adaxial side, no such features are seen, supporting the earlier X-ray studies of Kaiser et al. 1996 that measured P-gradients within leaf tissue.

IR-transmissive windows appropriate for isolating a portion of the root-zone for intense microscopic mapping are rare, but include ZnSe (20,000 - 454 cm-1) and KRS-5 (20,000 – 250 cm-1). Figure 3 shows the latest version of the "rhizobox", with a (50 mm x 20 mm, 2 mm thick) ZnSe window from Spectra-Tech. Deposited on the soil-facing side of the ZnSe window is a vapor-deposited gold spot for use as an internal IR reflection standard. Such a configuration was chosen to overcome any refractive effects from the IR window. A key disadvantage of the IR window materials listed above is their high index of refraction, 2.4 and 2.37 (measured at 2 m). In the IR wavelength range, uncoated windows will thus lose about 17% of the signal to reflections. While traditional salt-based IR windows have much lower loss figures, it may be appreciated that in the humid environment of roots, these windows would fog quickly. ZnSe and KRS-5 are also translucent in the visible range, which greatly aids in focusing the microscope.

Long-term experiments may include real-time analysis of soil enzymes (Lipson et al. 1998; Raab et al. 1999), and in this case, the discrete time-structure of the ALS beam allows collection of high-resolution FTIR scans of products. Results from this work will contribute substantially to NSF-sponsored soil ecological modeling studies being conducted at CU Boulder and UC

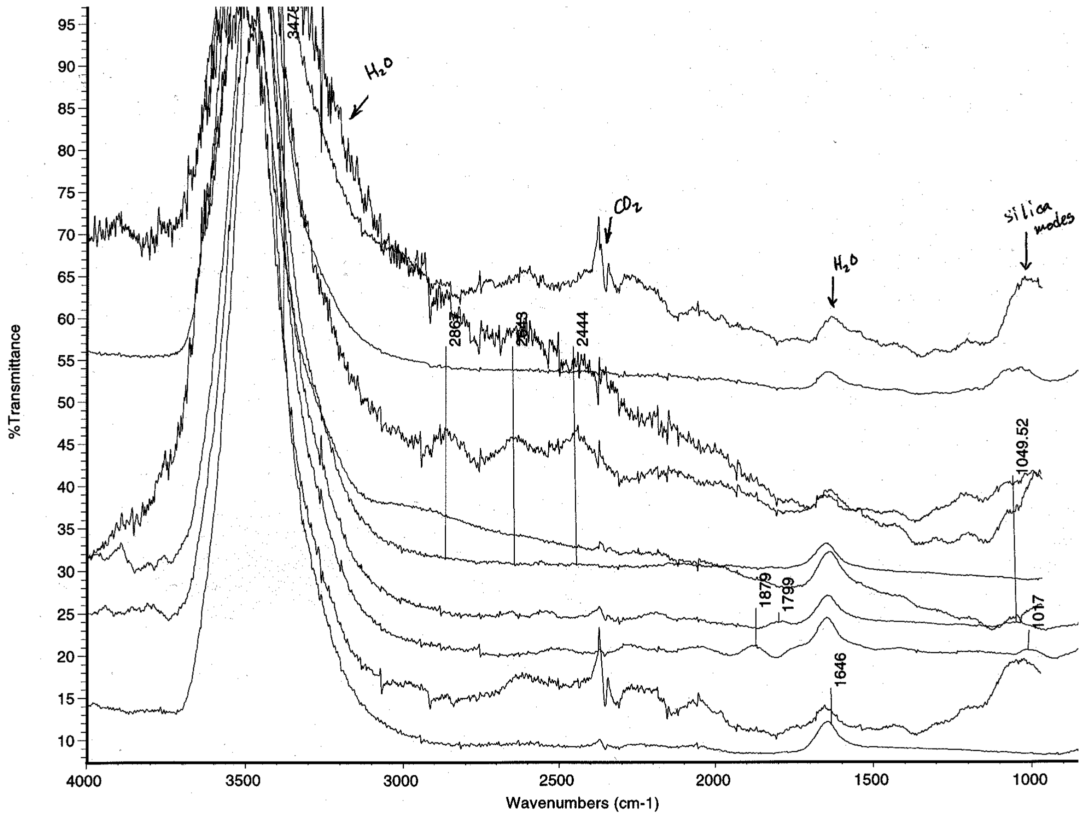

Figure 1.

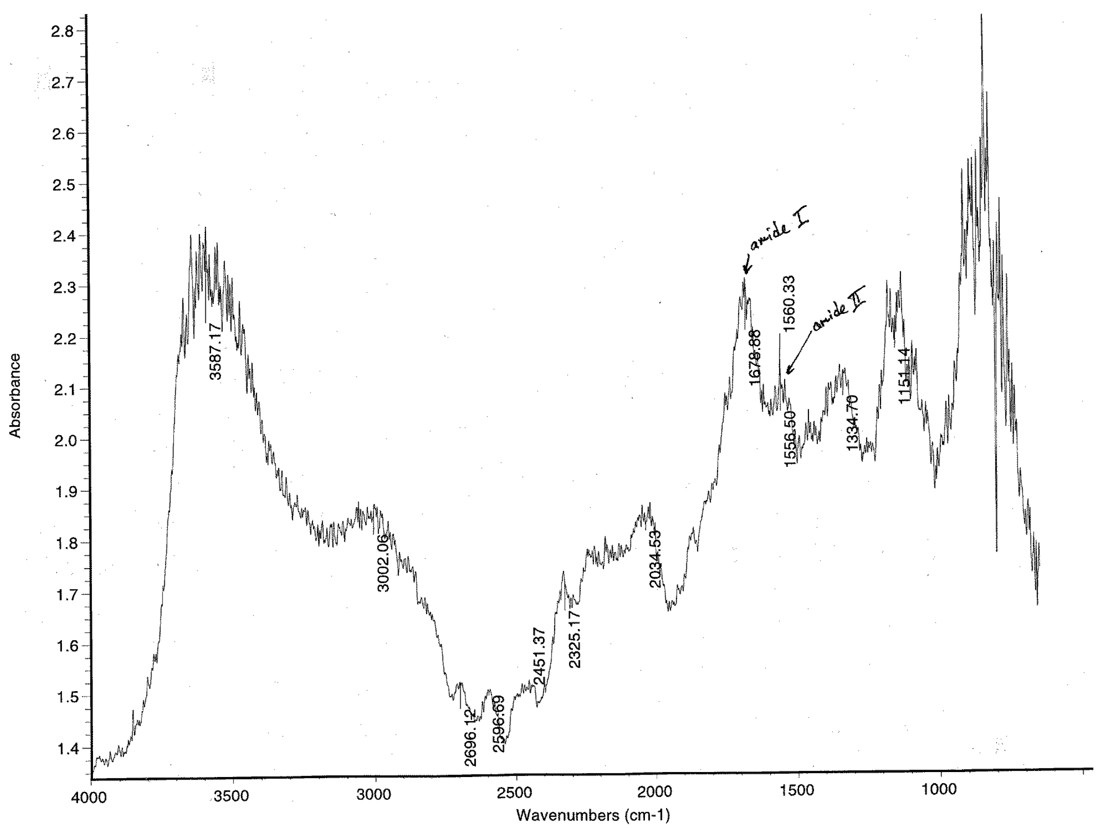

Figure 2.

Berkeley. These enzymes catalyze a variety of important bio-geochemical transformations of the global Carbon and Nitrogen cycle, and can affect pollution levels in the atmosphere.

Though formal experiments only began in late July of 1998, the support of Drs. Wayne McKinney and Michael Martin greatly accelerated my experiments at Beamline 1.4.3. The selection of IR window materials appropriate for a "rhizobox" (see Figure 3), and "hands-on" training in using the software for the IR microscope has allowed this project to generate the first IR spectral maps from intact plants using a Synchrotron IR facility.

Figure 3.

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